the mechanism of various diseases such as malignant hyperthermia, central core diseases, brody diseases and so on highly related with SR calcium abnormal handling. To overcome the limitation of reported genetically encoded Ca^{2+} sensors based on natural Ca^{2+} binding proteins of perturbing Ca^{2+} signaling, we report a novel design of calcium biosensor for the first time by rational de novo engineering a non-natural Ca^{2+} binding site into a single enhanced green fluorescent protein (EGFP), which can successfully quantitatively reveal the subcellular calcium signaling by fluorescence change. These developed Ca^{2+} sensors exhibit K_d values measured inside the mammalian cells in situ optimal for the measurement of Ca^{2+} in the SR. Metal selectivity of the sensors for Ca^{2+} in competition to excessive biological metal ions such as Mg^{2+}, K^+, Na^+ has been examined. In addition, these developed sensors can be targeted to the SR of muscle cells, and detected the Ca^{2+} signaling induced by various agonists and antagonists interacting with SR membrane Ca^{2+} pumps or receptors. Moreover, they exhibit fast response to Ca^{2+}. Further, their optical and conformational properties have been investigated using various spectroscopic methods, including high resonance resolution NMR. Moreover, more than 70% of the amino acids of the EGFP-based designed sensor have been successfully assigned using heteronuclear-labeled proteins. Our studies further reveal the key factors that contribute to the molecular mechanisms of the fluorescence change upon calcium binding and dynamic properties of our designed Ca^{2+} sensors.

1548-Pos

Orai1 Mediates Store-Operated Ca2+ Entry in Normal Skeletal Muscle and Exacerbated Ca2+ Entry in Dystrophic Muscle

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Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy, in which loss of dystrophin expression results in compromised sarcolemmal integrity. Although evidence shows that defects in Ca2+ homeostasis is a causal factor for the progressive cell death observed in DMD, the mechanism of Ca2+ deregulation is still under debate. Several laboratories showed that enhanced Ca2+ entry might serve as a pathological factor in dystrophic muscles. In this study, we explored the role of store operated Ca2+ entry (SOCE) in Ca2+ deregulation of dystrophic muscles. We used real-time PCR and Western blotting to detect known isoforms of Orai and STIM1 and determined that Orai1 was the most abundant in skeletal muscle and was significantly upregulated in muscles from mdx mice, while STIM1 levels remained largely unchanged. Furthermore, Mn2+ quenching of fura-2 fluorescence was applied to measure SOCE activity in flexor digitorum brevis (FDB) fibers and a significant increase in SOCE activity was detected in mdx fibers. Similar levels of resting [Ca]i was identified in wt and mdx groups, while peak response to C/R was significantly higher in mdx fibers than wt. Furthermore, we electroporated shRNA probe against mouse Orai1 into FDB muscle of living mice to produce effective knockdown (KD) of Orai1 expression. Two weeks after Orai1 KD, SOCE activity was eliminated in both wt and mdx muscle fibers and peak response to caffeine and ryanodine in mdx fiber sreturned to a level comparable to wt muscle fiber. Therefore, our study established that Orai1 is an essential component of SOCE machinery in adult skeletal muscle and indicates that Orai1-mediated SOCE could be the major pathway for additional Ca2+ entry into mdx muscle fibers, which would eventually lead to progression of DMD.

1549-Pos

Orai1 and STIM1 Mediate Capacitative Ca^{2+} Entry in Mouse Pulmonary Arterial Smooth Muscle Cells

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Previous studies in mouse pulmonary arterial smooth muscle cells (PASMCs) showed that TRPC1 and STIM1 mediates the sustained component of capacitative Ca^{2+} entry (CCE) but the molecular candidate(s) that mediate the transient component of CCE remains unknown. The aim of the present study was to further examine if Orai1 mediates the transient component of CCE through activation of STIM1 protein in mouse PASMCs.In primary cultured mouse PASMCs loaded with fura-2, cyclopiazonic acid (CPA) caused a transient followed by a sustained rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$).The transient but not the sustained rise in $[Ca^{2+}]_i$ was partially inhibited by nifedipine. The nifedipine-insensitive transient rise in $[Ca^{2+}]_i$ and the increase in Mn^{2+} quench of fura-2 fluorescence caused by CPA were both reduced in cells treated with Orai1 siRNA. These responses to CPA were further reduced in cells treated with Orai1 and STIM1 siRNA. Moreover, over-expression of STIM1 enhanced the rise in $[Ca^{2+}]_i$ and the increase in Mn^{2+} quench of fura-2 fluorescence caused by CPA and these responses were reduced in cells treated with Orai1 siRNA. RT-PCR revealed Orai1 and STIM1 mRNAs, and Western blot analysis identified Orai1 and STIM1 proteins in mouse PASMCs. Furthermore, Orai1 was found to co-immunoprecipitate with STIM1 and immunostaining showed co-localization of Orai1 and STIM1 proteins. These data provide direct evidence that the transient component of CCE is mediated by Orai1 channel through activation of STIM1 in mouse PASMCs. [Supported by HL49254, NCRR P20RR15581 (JR Hume) and AHA Scientist Development Grant (LC Ng)]

1550-Pos

In Smooth Muscle, Mitochondrial Movement is Restricted in Native Cells and Unrestricted and Trafficked When Cells are in Culture

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Positioning of mitochondria in the cell is important for the local provision of ATP and for regulation of $[Ca^{2+}]_c$ signals, lipid and reactive oxygen species production, redox control and initiation of cell death signals. In smooth muscle, mitochondrial Ca^{2+} uptake promotes Ca^{2+} release from the sarcoplasmic reticulum via by IP_3R, suggesting a localised removal of the ion from the IP_3R cytosolic face that maintains channel activity[1]. The close physical interaction that this relationship implies is not compatible, however, with the reported free movement of mitochondria throughout the cytosol. Here, image correlation based single particle tracking of mitochondria in freshly-isolated single smooth muscle cells from guinea-pig colon, shows that mitochondria displayed very limited movement. Brownian motion of mitochondria was detected but did not generate any significant displacement of the organelle over time. Neither the actin depolymerising agent latrunculin B (10 μM), nor the microtubule disrupter nocodazole (10 μM) increased mitochondrial movement. In contrast, when freshly-isolated smooth muscle cells were maintained in cell culture conditions for 14 days mitochondrial motility was substantially increased. Mitochondria displayed rapid, directed motion and Brownian movement resulted in displacement of the mitochondria over time. These results suggest that in freshly-isolated smooth muscle cells, mitochondria are either confined or tethered to limit movement; whereas when the same cells divide and proliferate in culture these restraints are lost, mitochondria display random-walk diffusive motion and are accessible to the intracellular trafficking machinery.

1. Chalmers S & McCarron JG (2008) J Cell Sci. 121:75-85.
2. Saunter CD *et al.* (2009) FEBS Lett. 583:1267-73.

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1551-Pos

Synchronization of Waveform Analysis with Monitoring of Localized [Ca2+] in the Beating Flagellum of Single Sperm

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Much past work indicates that in mammalian sperm Ca^{2+} is the messenger that controls waveform flagellar symmetry, and that the cAMP messenger controls beat frequency. We have now constructed an imaging system that uses dual pulsed-LED sources and records interleaved stop-motion brightfield and fluorescence images of individual loosely-tethered mouse sperm loaded with the Ca^{2+} probe fluo-4. The brightfield images report flagellar beat frequencies of 2-4 hertz for resting sperm, and the fluo-4 images report similar $[Ca^{2+}]$ (~150 nM) in the head, the midpiece, and the cytoplasmic droplet located at the flagellar midpiece/principal-piece junction. Stimulation (10-20s) by local perfusion with alkaline-depolarizing medium K8.6 raises $[Ca^{2+}]$ ~4-fold in each region. The $[Ca^{2+}]$ rises first in the droplet, then the midpiece, and finally in the head. Recovery towards baseline is slow ($t_{1/2}$>30s). Analysis of the waveform shows that increases in flagellar beat asymmetry accompany the increased $[Ca^{2+}]$ but that beat frequency remains unchanged before, during, and after stimulus. The delayed Ca^{2+} responses in midpiece and head are consistent with evoked localized entry through CatSper ion channels in the principal piece with subsequent diffusional redistribution. The accompanying increases in beat asymmetry without increases in frequency suggests that evoked Ca^{2+} entry does not engage cAMP-mediated signaling in sperm.

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